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Mineralocorticoid Receptor Antagonism Ameliorates Left Ventricular Diastolic Dysfunction and Myocardial Fibrosis in Mildly Symptomatic Patients With Idiopathic Dilated Cardiomyopathy

A Pilot Study

Hideo Izawa, MD, PhD; Toyoaki Murohara, MD, PhD; Kohzo Nagata, MD, PhD; Satoshi Isobe, MD, PhD; Hiroyuki Asano, MD; Tetsuya Amano, MD, PhD; Sahoko Ichihara, MD, PhD; Tomoko Kato, MD, PhD; Satoru Ohshima, MD; Yosuke Murase, MD; Shigeo Iino, MD, PhD; Koji Obata, PhD; Akiko Noda, PhD; Kenji Okumura, MD, PhD; Mitsuhiro Yokota, MD, PhD

Background—Mineralocorticoid receptor antagonism reduces mortality associated with heart failure by mechanisms that remain unclear. The effects of the mineralocorticoid receptor antagonist spironolactone on left ventricular (LV) function and chamber stiffness associated with myocardial fibrosis were investigated in mildly symptomatic patients with idiopathic dilated cardiomyopathy (DCM).

Methods and Results—Twenty-five DCM patients with a New York Heart Association functional class of I or II were examined before and after treatment with spironolactone for 12 months. LV pressures and volumes were measured simultaneously, and LV endomyocardial biopsy specimens were obtained. Serum concentrations of the carboxyl-terminal propeptide (PIP) and carboxyl-terminal telopeptide (CITP) of collagen type I were measured. The patients were divided into 2 groups on the basis of the serum PIP/CITP ratio ($\leq$35, group A, n=12; >35, group B, n=13), an index of myocardial collagen accumulation. LV diastolic chamber stiffness, the collagen volume fraction, and abundance of collagen type I and III mRNAs in biopsy tissue were greater and the LV early diastolic strain rate (tissue Doppler echocardiography) was smaller in group B than in group A at baseline. These differences and the difference in PIP/CITP were greatly reduced after treatment of patients in group B with spironolactone, with treatment having no effect on these parameters in group A. The collagen volume fraction was significantly correlated with PIP/CITP, LV early diastolic strain rate, and LV diastolic chamber stiffness for all patients before and after treatment with spironolactone.

Conclusions—Spironolactone ameliorated LV diastolic dysfunction and reduced chamber stiffness in association with regression of myocardial fibrosis in mildly symptomatic patients with DCM. These effects appeared limited, however, to patients with increased myocardial collagen accumulation. **(*Circulation*. 2005;112:2940-2945.)**

Key Words: biopsy ■ cardiomyopathy ■ collagen ■ drugs ■ heart failure

Dilated cardiomyopathy (DCM) is characterized by progressive left ventricular (LV) dilation and greatly impaired LV systolic function, eventually culminating in end-stage congestive heart failure and death.[1,2] Despite recent improvements in medical management, the overall prognosis of individuals with DCM is still poor. The Randomized Aldactone Evaluation Study (RALES) demonstrated a 30% reduction in the mortality of patients with a New York Heart Association (NYHA) functional class of III or IV after treatment with the mineralocorticoid receptor antagonist spironolactone in addition to standard therapy for congestive heart failure.[3] More recently, treatment with another mineralocorticoid receptor antagonist, eplerenone, was found to reduce cardiovascular mortality by 17% in patients with post–acute myocardial infarction.[4] However, the possible therapeutic efficacy of mineralocorticoid receptor antagonists in patients with mildly symptomatic heart failure such as those with an NYHA functional class of I or II has not been previously examined.

The mechanisms of the beneficial effect of mineralocorticoid receptor antagonism in patients with heart failure also remain to be fully elucidated. Given that aldosterone pro-

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From the Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya (H.I., T.M., S. Isobe, H.A., T.K., S.O., Y.M., S. Iino, K. Okumura); Nagoya University School of Health Sciences, Nagoya (K.N., A.N.); Department of Cardiovascular Genome Science, Nagoya University School of Medicine, Nagoya (K. Obata, M.Y.); and Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu (S. Ichihara), Japan.

Correspondence to Hideo Izawa, MD, PhD, Department of Cardiology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466–8550, Japan. E-mail izawa@med.nagoya-u.ac.jp

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motes myocardial fibrosis in animals and humans,[5–9] one of the underlying mechanisms of the therapeutic effect of mineralocorticoid receptor antagonism in chronic heart failure is thought to be prevention of this action of aldosterone.[10] Although mineralocorticoid receptor antagonism was found to induce a marked reduction in the serum concentrations of markers of myocardial fibrosis in patients with chronic heart failure, direct clinical evidence of regression of myocardial fibrosis induced by such treatment has been lacking.[11,12] Regression of myocardial fibrosis in response to treatment with a mineralocorticoid receptor antagonist would be expected to result in amelioration of LV diastolic dysfunction, which has been commonly detected in patients with DCM by Doppler echocardiography, especially tissue Doppler imaging.[13,14]

We have now investigated whether treatment with the mineralocorticoid receptor antagonist spironolactone induces regression of myocardial fibrosis in DCM patients with an NYHA functional class of I or II by 3 independent methods: histomorphometric assessment of myocardial collagen content (collagen volume fraction [CVF]), examination of the expression of collagen types I and III, and biochemical measurement of myocardial collagen accumulation. We also determined whether regression of myocardial fibrosis is associated with improvement in LV diastolic function.

Methods

Patients

The study protocol was approved by the Ethics Review Board of the Nagoya University School of Medicine, and written informed consent was obtained from each patient before entry into the study. The study population comprised 25 ambulatory patients with idiopathic DCM (19 men, 6 women) and a mean age of 49 years (range, 27 to 65 years). The patients included 17 individuals with an NYHA functional class of I and 8 with class II. All patients had previously been admitted to hospital as a result of congestive heart failure, but they had been in a stable condition for a mean of 10±4 months (range, 6 to 21 months) before enrollment in the study. DCM was defined by an LV ejection fraction of <45% (as determined by contrast ventriculography) in the absence of coronary artery stenosis >50% (as determined by coronary angiography), valvular heart disease, arterial hypertension, or cardiac muscle disease secondary to any known systemic condition.[15] Patients with atrial fibrillation were excluded from the study to facilitate the evaluation of diastolic function. Individuals with conditions associated with changes in the serum concentrations of markers of myocardial fibrosis (connective tissue disorders, pulmonary fibrosis, liver cirrhosis, osteoporosis, renal insufficiency, malignancy at any site) also were excluded. All study patients had been treated with both ACE inhibitors and loop diuretics for >3 months at the baseline condition. No patients had received β-blockers or angiotensin II receptor blockers.

Study Protocol

Physical examination, laboratory measurements, echocardiography, and LV catheterization, including determination of LV pressure with a micromanometer-tipped catheter and LV endomyocardial biopsy, were performed at baseline for all patients. The patients were then treated with spironolactone (25 mg/d) in addition to their existing drug regimen for 12 months, after which they were subjected to the same procedures as performed at baseline.

Laboratory Measurements

Peripheral venous blood samples were collected from patients after they had been in the supine position for 30 minutes. Serum was prepared and stored at −80°C until analysis. The serum concentrations of the carboxyl-terminal propeptide of procollagen type I (PIP), the carboxyl-terminal telopeptide of collagen type I (CITP), and the amino-terminal propeptide of procollagen type III (PIIINP) were measured with radioimmunoassay kits as markers of collagen type I synthesis, collagen type I degradation, and collagen type III synthesis, respectively. The lower detection limits of the assays were 1.2 μg/mL for PIP, 0.5 μg/mL for CITP, and 0.2 U/mL for PIIINP. The PIP/CITP ratio, an index of coupling between the synthesis and degradation of collagen type I, also was calculated as a serum marker of myocardial collagen accumulation.[16] The serum concentration of brain natriuretic peptide (BNP) also was determined with a radioimmunoassay kit.

Echocardiography

We performed M-mode, 2D, pulsed Doppler and tissue color Doppler echocardiography with a phased-array electronic ultrasound system (Vivid Seven, GE VingMed Ultrasound). The peak flow velocities at the mitral level during rapid filling (E) and during atrial contraction (A), as well as the E/A ratio, were calculated from the pulsed Doppler echocardiographic data. For tissue color Doppler imaging, scanning was performed longitudinally from the apex to acquire the 4-chamber view. The LV myocardium was divided into 8 segments (anterior base, anterior apex, inferior base, inferior apex, septal base, septal apex, lateral base, lateral apex), and longitudinal strain and strain rate were estimated for each segment by measuring the spatial velocity gradient over a computation area of 8 to 10 mm^2.[17] The LV early diastolic strain rate, a relatively load-independent estimate of LV diastolic function,[18] was then calculated from the average strain and strain rate. All echocardiographic evaluations were performed by an operator blinded to the results of other examinations.

Catheterization

An externally balanced and calibrated 6F pigtail angiographic micromanometer-tipped catheter was advanced into the left ventricle for measurement of LV pressure. We evaluated the maximum first derivative of LV pressure (LV dP/dt_{max}) as an index of contractility and the pressure half-time ($T_{1/2}$) as an index of relaxation, as previously described.[19] Left ventriculography was performed immediately after measurement of LV pressure, and LV volume was calculated by the area-length method.

Quantitative Morphometry

LV endomyocardial biopsy specimens were obtained with a 6F bioptome. The tissue was fixed immediately in 10% buffered formalin and embedded in paraffin. Three or 4 specimens were analyzed for each patient. Tissue sections were stained with the collagen-specific dye picrosirius red. Using an automated image analysis system (Win ROOF 5.0, Mitani), we calculated the CVF as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas in all fields analyzed for each section.[20] These histological evaluations were performed without knowledge of which patient provided the tissue sections.

Quantitative Reverse-Transcription Polymerase Chain Reaction Analysis

Total RNA was isolated from 1 to 2.5 mg frozen LV biopsy specimens and subjected to quantitative reverse transcription and polymerase chain reaction (PCR) analysis as previously described.[21] The amounts of the mRNAs for collagen types I and III were thus determined with a fluorogenic 5′-nuclease PCR assay and an ABI PRISM 7700 sequence detector (Perkin-Elmer). All PCR assays were performed in triplicate. TaqMan control reagents (Perkin-Elmer) were used to detect human GAPDH mRNA as an internal standard.

Statistical Analysis

Data are presented as mean±SD. The baseline characteristics of 2 groups of patients were compared by Student's *t* test for unpaired

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TABLE 1. Clinical Characteristics of Study Subjects and Comparison of Hemodynamic Parameters Between Baseline and After Treatment With Spironolactone

	Group A		Group B	
	Baseline	After Treatment	Baseline	After Treatment
Gender, M/F	9/3		10/3	
Age, y	47±11		50±9	
NYHA functional class, I/II	9/3	11/1	8/5	11/2
Heart rate, bpm	72±7	71±7	73±9	72±10
SBP, mm Hg	120±16	120±10	117±13	116±14
DBP, mm Hg	78±6	76±6	77±6	76±6
Wall thickness, mm	7±2	7±2	7±1	7±2
LV end-diastolic diameter, mm	62±9	56±5*	63±10	60±7*†
LVEF, %	33±5	34±7	33±8	36±8
LVEDV, mL	184±29	168±12*	195±20	180±17*†
LVEDP, mm Hg	19±3	17±2*	22±2†	19±2*†
LVEDP/LVEDV, mm Hg/mL	0.102±0.010	0.100±0.008	0.116±0.010†	0.107±0.010*†
LV dP/dt_{max}, mm Hg/s	1140±347	1272±377*	1031±243	1108±238*
$T_{1/2}$, ms	42±6	39±4*	46±6†	41±6*
E/A	0.83±0.16	0.89±0.15	0.78±0.14	0.81±0.08
LV early diastolic strain rate, 1/s	0.78±0.16	0.80±0.22	0.51±0.13†	0.66±0.10*†

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; and LV EF, LV ejection fraction. Data are mean±SD.

*$P<0.05$ vs corresponding baseline value; †$P<0.05$ vs corresponding value for group A.

data or the χ^2 test for categorical data. The effects of spironolactone treatment for 12 months were evaluated with Student's t test for paired data. The relation between continuous variables was assessed by linear regression analysis. A value of $P<0.05$ was considered statistically significant.

Results

No adverse effects or complications occurred during treatment of the study patients with spironolactone, and all subjects completed the study protocol. We divided the patients into 2 groups on the basis of the biochemical index of coupling between the synthesis and degradation of collagen type I at baseline: 12 patients with a serum PIP/CITP ratio of ≤35 (group A) and 13 patients with a corresponding value of >35 (group B). A PIP/CITP ratio of 35 corresponded to the median value of this parameter and thus was used as the cutoff to separate the 2 groups.

Clinical and Hemodynamic Characteristics Before and After Treatment

There were no significant differences in age, gender, or NYHA functional class between groups A and B, and heart rate, LV ejection fraction, LV dP/dt_{max}, and the E/A ratio at baseline did not differ significantly between the 2 groups (Table 1). In contrast, the ratio of LV end-diastolic pressure to LV end-diastolic volume (LVEDP/LVEDV), an indicator of LV diastolic chamber stiffness, was significantly increased at baseline in group B compared with group A. The $T_{1/2}$ at baseline was significantly greater and LV early diastolic strain rate was significantly smaller in group B than in group

TABLE 2. Comparison of Biochemical Parameters Between Before and After Treatment With Spironolactone

	Group A		Group B	
	Baseline	After Treatment	Baseline	After Treatment
PIP, μg/mL	68.3±10.0	64.6±10.6	85.9±6.6†	75.0±4.6*†
CITP, μg/mL	3.35±1.04	3.52±0.84	2.09±0.35†	2.75±0.59*†
PIP/CITP	21.9±6.6	19.1±4.5	42.0±6.8†	28.3±5.6*†
PIIINP, U/mL	0.45±0.14	0.41±0.08	0.59±0.20†	0.44±0.18*
Aldosterone, pg/mL	186±37	222±151	161±35	189±41*
Norepinephrine, pg/mL	294±120	271±109	340±108	282±95
BNP, pg/mL	92±46	77±42*	133±44†	88±37*

Data are mean±SD.

*$P<0.05$ vs corresponding baseline value; †$P<0.05$ vs corresponding value for group A.

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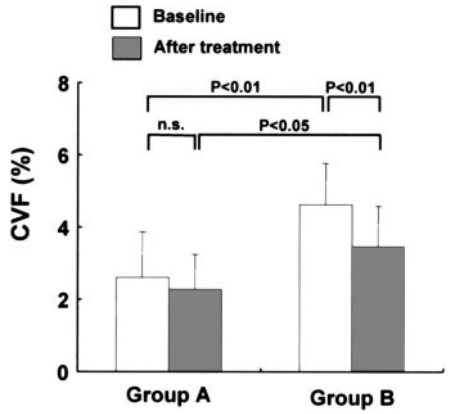

Figure 1. CVF values at baseline and after treatment with spironolactone for patients in groups A and B. Data are mean±SD.

A. After treatment with spironolactone, LV dP/dt_{max} was significantly increased although LVEDV was significantly decreased in both groups. $T_{1/2}$ was also significantly decreased in both groups after treatment. LVEDP/LVEDV was reduced and LV early diastolic strain rate was increased in group B after treatment with spironolactone, whereas no significant changes in these variables were apparent in group A.

Biochemical and Histological Parameters Before and After Treatment

The serum concentrations of PIP (a marker of collagen type I synthesis), PIIINP (a marker of collagen type III synthesis), and BNP were significantly higher and that of CITP (a marker of collagen type I degradation) was significantly lower at baseline in group B than in group A (Table 2). Marked perivascular and interstitial fibrosis, as revealed by staining with the collagen-specific dye picrosirius red, was detected in the endomyocardial biopsy specimens obtained at baseline from patients in group B but not in those from patients of group A (data not shown). The CVF at baseline was also significantly greater in group B than in group A (Figure 1). The extent of perivascular and interstitial fibrosis and the CVF in patients of group B were reduced after treatment with spironolactone, and these effects were accompanied by a significant reduction in the PIP/CITP ratio and the serum

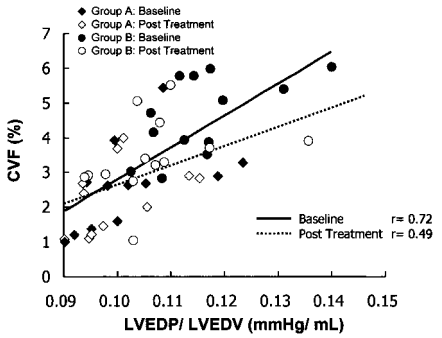

Figure 5. Correlation between CVF and LV diastolic chamber stiffness at baseline and after treatment with spironolactone for all patients.

levels of PIP, PIIINP, and BNP, as well as by a significant increase in that of CITP.

Expression of Collagen Types I and III Before and After Treatment

The amounts of collagen type I and III mRNAs in endomyocardial tissue at baseline were significantly higher in group B than in group A (Figure 2). Those in group B, but not those in group A, were significantly reduced after treatment with spironolactone, consistent with the biochemical and histological findings.

Relations Between CVF and Myocardial Collagen Accumulation, LV Diastolic Function, and LV Diastolic Chamber Stiffness

The CVF was significantly correlated with the serum PIP/CITP ratio (Figure 3), LV early diastolic strain rate (Figure 4), and LVEDP/LVEDV (Figure 5) values obtained before and after treatment with spironolactone in all patients.

Discussion

We have evaluated the effects of treatment with the mineralocorticoid receptor antagonist spironolactone on LV diastolic function and myocardial collagen content in mildly symptomatic patients with DCM (NYHA functional class I or II). The main findings of our study include the following: (1) The PIP/CITP ratio in serum, a marker of myocardial colla-

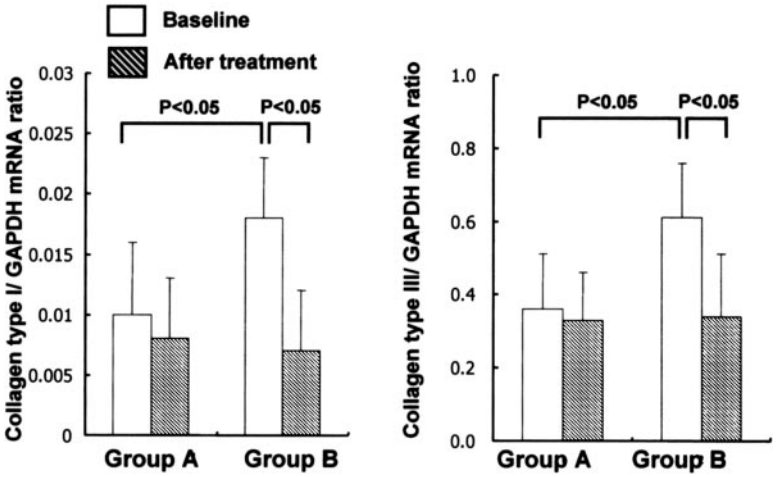

Figure 2. Reverse-transcription PCR analysis of the abundance of collagen type I (left) and type III (right) mRNAs in endomyocardial biopsy specimens obtained from patients in groups A and B at baseline and after treatment with spironolactone. Data are normalized by the amount of GAPDH mRNA and are mean±SD.

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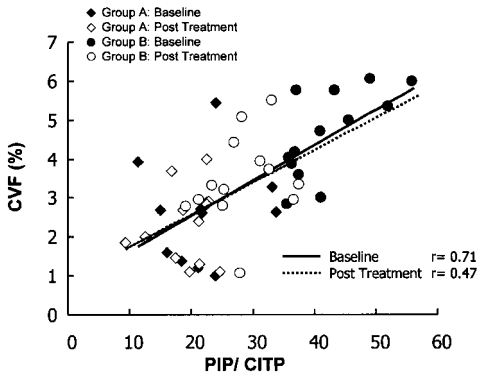

Figure 3. Correlation between CVF and PIP/CITP values at baseline and after treatment with spironolactone for all patients.

gen accumulation, was significantly correlated with myocardial collagen content as assessed by histomorphometric analysis; (2) LV diastolic function was inversely related to myocardial collagen content; (3) spironolactone induced both a marked reduction in the extent of myocardial fibrosis and a significant improvement in LV diastolic function, and these effects were accompanied by a reduction in the PIP/CITP ratio; (4) the effects of spironolactone were limited to patients with an increased PIP/CITP ratio; and (5) spironolactone induced a significant improvement in LV systolic function (as evaluated by LV dP/dt_{max}) and in LV relaxation (as evaluated by $T_{1/2}$) in all patients.

Serum Levels of Procollagen Peptide Fragments Are Markers of Collagen Turnover

The PIP fragment is released during the extracellular processing of procollagen type I before the formation of collagen fibers and serves as a marker of fibrogenesis.[22] CITP is a pyridinoline–cross-linked telopeptide produced as a result of the hydrolysis of collagen type I fibrils by matrix metalloproteinase-1 and is a marker of collagen type I degradation.[23] The PIP/CITP ratio, an index of coupling between the synthesis and degradation of collagen type I, was found to be higher in hypertensive patients with increased collagen accumulation in myocardial tissue than in those with normal collagen accumulation.[20] The PIP/CITP ratio thus serves as a serum marker of myocardial collagen accumulation. Indeed, both the CVF and the abundance of collagen type I and III mRNAs in the LV myocardium were higher in the patients with an increased PIP/CITP ratio (group B) than in those with a lower PIP/CITP ratio (group A) in the present study.

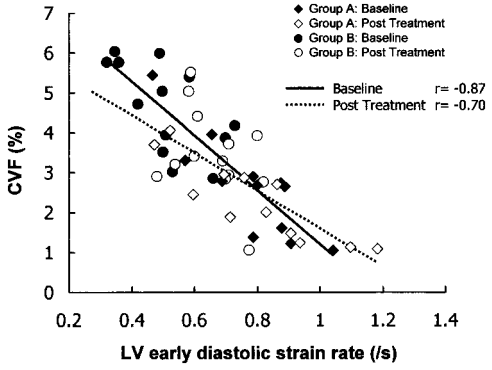

Figure 4. Correlation between CVF and LV early diastolic strain rate at baseline and after treatment with spironolactone for all patients.

The plasma concentration of aldosterone correlates with mortality in individuals with chronic heart failure.[24] We have now shown that treatment with the mineralocorticoid receptor antagonist spironolactone induced both a substantial regression of myocardial fibrosis and a significant amelioration of LV diastolic dysfunction in patients with an elevated serum marker of myocardial collagen accumulation. In addition, myocardial collagen content was strongly correlated with LV diastolic function in all patients before and after treatment with spironolactone. We therefore propose that the ability of spironolactone to improve LV diastolic function in mildly symptomatic patients with DCM is related to its capacity to reduce the extent of myocardial fibrosis through normalization of collagen metabolism. This scenario might explain the previous finding that the beneficial effect of spironolactone on survival and hospitalization in RALES was significant only in patients with higher levels of serum markers of collagen synthesis.[11]

LV diastolic dysfunction is an important prognostic marker in patients with chronic heart failure.[25] The efficacy of mineralocorticoid receptor antagonism with regard to mortality reduction in patients with heart failure might thus be due to the improvement in LV diastolic function induced by such treatment. Our present results and those of the previous study by Zannad et al[11] suggest that such an effect of spironolactone might be limited to patients with increased collagen accumulation in myocardial tissue. Indeed, the serum concentration of BNP, which is also an important prognostic marker in chronic heart failure,[26] was reduced by treatment with spironolactone to a greater extent in patients with an increased serum PIP/CITP ratio at baseline than in those with a lower PIP/CITP ratio.

Although a marked effect of spironolactone on myocardial fibrosis was evident in the present study, the number of patients in this pilot study was small. In addition, our study included only patients with a nonischemic origin, and none of the subjects had been treated with β-blockers. Further studies with larger numbers of patients, including those with an ischemic origin and those treated with β-blockers, are needed before our results can be applied more generally to mildly symptomatic patients with heart failure.

In summary, we have shown that treatment with the mineralocorticoid receptor antagonist spironolactone resulted in an improvement in LV diastolic function that was associated with regression of myocardial fibrosis in mildly symptomatic patients with DCM. This effect of spironolactone, however, was limited to patients with increased myocardial collagen accumulation. Our data suggest that collagen accumulation that results from mineralocorticoid receptor stimulation might play a critical role in the impairment of LV diastolic function in patients with DCM.

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